The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Influence of serum leptin levels and Q223R leptin receptor polymorphism on clinical characteristic of patients with rheumatoid arthritis from Western Mexico

Luis I. Angel-Chávez[1], Elizabeth Ruelas-Cinco[2], Jorge Hernández-Bello[2], Elena Castro[3], Mirna Vázquez-Villamar[4], Isela Parra-Rojas[4], L. Michele Brennan-Bourdon[2], Salvador Muñoz-Barrios[2], Celia Guerrero-Velázquez[2], José Francisco Muñoz-Valle[2]

[1] *Instituto de Ciencias Biomédicas, Departamento de Ciencias de la Salud, Universidad Autónoma de Ciudad Juárez, Mexico*

[2] *Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Mexico*

[3] *Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Mexico*

[4] *Unidad Académica de Ciencias Químico-Biológicas, Universidad Autónoma de Guerrero, Mexico*

ARTICLE INFO

Corresponding author:
José Francisco Muñoz-Valle
Insurgentes 244-1
Colonia Lomas de Atemajac
C.P. 45178
Zapopan, Jalisco
Mexico
Phone: +52 3338540154
E-mail: biologiamolecular@hotmail.com

Key words:
leptin receptor, leptin, rheumatoid arthritis, polymorphism, Q223R

ABSTRACT

Objective

The aim of the present study was to evaluate the possible association between the Q223R Leptin receptor (*LEPR*) polymorphism (A>G; rs1137101) and leptin levels in patients with rheumatoid arthritis (RA) from Western Mexico.

Methods

A cross-sectional study was performed with 70 RA patients and 74 controls subject (CS). Disease activity was evaluated using DAS28 score, the Q223R *LEPR* polymorphism was determined by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and serum leptin levels, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF) were quantified.

Results

RA patients had significant high serum leptin levels compared with CS; leptin levels correlated strongly with body composition measures, but not with inflammatory markers, disease evolution, and activity. The genotype and allele frequencies of the Q223R *LEPR* polymorphism were not associated with RA. Similarly, leptin levels did not differ between Q223R *LEPR* genotypes.

Conclusion

The *LEPR* Q223R polymorphism was not associated with RA risk in patients from Mexican population, even though high levels of serum leptin were present and these could explain the low weight observed in RA patients when they were compared to control subjects. However, the serum leptin levels did not correlate with inflammatory markers, severity and disease evolution.

❖❖❖❖❖

INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common inflammatory rheumatic disease in which joints of the hand and feet are mainly affected (1). One of the main features in the pathophysiology of this disease is the production of inflammatory cytokines in the synovial membrane and the subsequent joint damage.

Leptin is a cytokine that mediates both immune cell recruitment and complex intracellular signaling control mechanisms that characterize inflammation, thus it may have a potential role in RA. Several studies have reported a two to three times elevation in serum leptin levels in women as compared to men (2,3), furthermore, serum leptin levels are high in RA patients as compared to controls (4-11). Leptin levels also correlate with disease activity (5-7,9) and pro-inflammatory activity (12).

In 1994, it was discovered that leptin is produced by adipocytes and is crucial for the control of appetite (13). This hormone exhibits a structural similarity with the Granulocyte Colony-Stimulating Factor (G-CSF) and with a number of cytokines including Interleukin 6 (IL-6), Ciliary Neurotrophic Factor (CNTF) and Leukemia Inhibitory Factor (LIF) (14,15).

In humans, blood leptin levels are proportional to the amount of body fat (16). At the level of the Central Nervous System (CNS) leptin controls energy expenditure (17), whereas in the periphery it regulates endocrinal function, reproduction, and immunity (18).

Leptin acts by binding to specific receptors present on cell membranes. This receptor is expressed in many tissues in multiple isoforms, resulting from a "splicing" of its alternate mRNA.

The different isoforms of the receptor can be classified as long (OB-Rb), short (OB-Ra, OB-Rc, and OB-Rd) and soluble (OB-Re) (19). Considering the multiple isoforms, it is possible to hypothesize the possibility that leptin exerts multiple effects on tissues.

Several single nucleotide polymorphisms and mutations of leptin receptor (*LEPR*) gene have been linked to diseases accompanying obesity and/or obesity-related diseases in different populations (20-24), nonetheless there are very few studies evaluating their association with RA.

As far, three missense variants Q223R, K109R, K656N in the *LEPR* gene have been described, and their associations with adiposity examined. In most cases, the *LEPR* Q223R polymorphism has been associated with high serum leptin levels; this variant is a substitution of G>A at 668 position, a putative leptin binding region (20,21).

The aim of the present study was to evaluate the possible association between the Q223R *LEPR* polymorphism and leptin levels in patients with RA from Western Mexico.

MATERIALS AND METHODS

A cross-sectional study was performed with a Mexican mestizo population of 70 RA patients and 74 Control subjects (CS) from Guadalajara, State of Jalisco, Mexico. The diagnosis of RA was based on the American College of Rheumatology criteria and the patients were selected from an outpatient rheumatology clinic from the public healthcare system. The CS were randomly selected from an open population sample. The study was approved by the Investigation, Ethics and Biosecurity Committee of the Health Sciences University Center of the University of Guadalajara. All participants gave informed consent prior to inclusion in the study; the study was carried out in accordance with the principles of the World Medical Association's Declaration of Helsinki.

Following blood sampling, serum leptin levels were determined, DNA was extracted and *LEPR* genotypes were identified. Additionally, a detailed clinical history was collected from each participant.

Leptin levels were quantified using a solid phase enzyme-linked immunosorbent assay (ELISA) (DRG International, Inc., USA). The erythrocyte sedimentation rate (ESR) was determined using the Westergren method. The C-reactive protein (CRP) and rheumatoid factor (RF) were quantified using Immage™ Immunochemistry system (Beckman Coulter System).

The Miller method was used to extract DNA. The DNA pellet was resuspended in 200 µL of sterile water, and concentration and integrity were calculated by spectrophotometry. The Q223R variants of the *LEPR* gene were determined by PCR-RFLP technique, as described previously (25).

Descriptive statistics (mean and standard deviation or median and percentile 5-95) were used to describe participant characteristics, additional statistical analysis was performed following data normality check by the Kolmogorov-Smirnov test.

Chi-square test was used to evaluate the statistical association of the polymorphism. Odds Ratio (OR) was calculated for the evaluation of polymorphism as a risk factor associated with rheumatoid arthritis.

The Student *t* or Mann-Whitney test was used to evaluate mean or median differences between the two study groups. One-way ANOVA with Bonferroni *post-hoc* tests were used to evaluate the mean differences between the genotypes of the *LEPR* gene.

Pearson or Spearman correlations were used to evaluate the correlation between leptin levels with age, weight, height, Body Mass Index (BMI), CRP, RF, DAS28 and disease duration. Statistical significance was reported when p values were below 0.05. Data analysis was carried out with the SPSS version 19.0 statistical package (SPSS, Inc., Chicago, IL).

RESULTS

The clinical characteristics of the study subjects are shown in Table 1. A total of 70 RA patients were enrolled, 4 were males and 66 females, with an age mean of 48±13 years and disease duration of 7.5 years, the clinical activity according to the DAS28 score was 5.1±1.6. A total of 74 CS were selected, 24 were male and 50 females, with a mean age of 37±12 years. Upon comparing the two groups, there was no statistically significant difference in height and BMI (p>0.05, data not shown), and there was a significant difference in age, weight, inflammation markers (CRP and ESR) and RF (p<0.05, data not shown).

Table 1 Clinical characteristics of the study groups

Variables	RA (n= 70)	CS (n=74)
Age (years) [c]	48 ± 13	37 ± 12
Sex (male/female) [b]	4 / 66	24/50
Weight (kg) [a]	60.2 (41.1 - 90.3)	67.35 (51-94)
Height (m) [c]	1.55 ± 0.1	1.64 ± 0.1
BMI (kg/m^2) [c]	25.8 ± 5.1	25.9 ± 4.5
Disease status		
Duration of disease (years) [a]	7.5 (0.5-30)	
DAS28 [c]	5.1 ± 1.6	
Clinical assessment		
Leukocytes (X10^9/L) [c]	6.2 ± 1.8	6.4 ± 1.6
CRP (mg/dL) [a]	2.3 (0.3-40.8)	0.2 (0.1-0.9)
ESR (mm/h) [c]	37 ± 14	18.9 ± 11.7
Rheumatoid factor (UI/mL) [a]	258.5 (20-3426)	20 (20-21.4)

[a] *Data provided in median (p5–p95)*
[b] *Data provided in n*
[c] *Data provided in mean ± SD*
RA: rheumatoid arthritis; CS: control subject; ESR: erythrocyte sedimentation Rate; CRP: C reactive protein; DAS-28: disease activity score 28. Normal distribution was determined using Kolmogorov-Smirnov test.

Genotypes and allele frequencies of the *LEPR* Q223R polymorphism are shown in Table 2 and they were in Hardy-Weinberg equilibrium (χ^2 0.08, p=0.77). When we evaluated the genotypes, no causal or statistical association was found between the RA patients and the control subjects.

Serum leptin levels were elevated in patients with RA as compared to CS (12.4 (1.7-48.5) ng/mL *vs* 9.4 (1.4-29.9) ng/mL, p=0.03) (Figure 1).

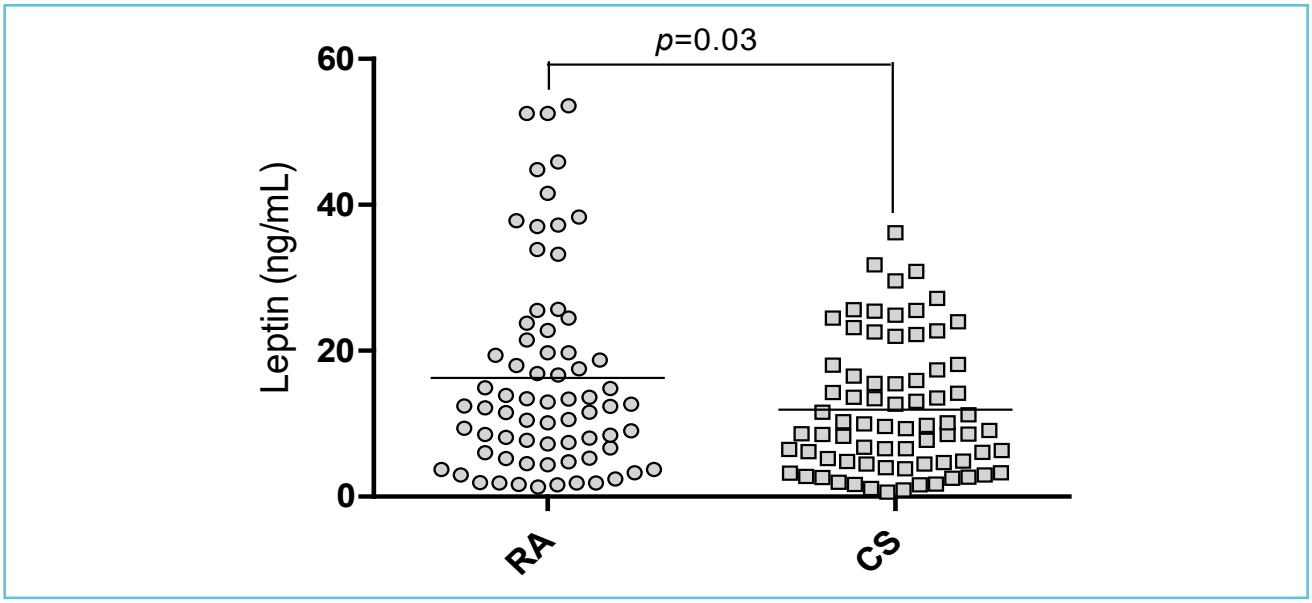
When the serum leptin levels were stratified by genotype in RA patients group, the carriers of the AA genotype had a median of 13.4 (1.8-45.6) ng/mL, while carriers of the AG and GG genotypes had a median of 11.8 (1.6-52.5) ng/mL and 9.9 (2.4-52.5) ng/mL, respectively (Figure 2A). In the CS group, carriers of the AA genotype had a leptin median of 6.34 (0.7-23.5) ng/mL, while the carriers of the AG and GG genotypes had a median of 10.1 (1.5-31) ng/mL and 11.7 (1.6-36.1) ng/mL, respectively (Figure 2B).

Table 2 Genotypic distribution and allelic frequencies of Q223R polymorphism

SNP	Genotype	RA (n=70) % (n)	CS (n = 74) % (n)	*p value*[a]	OR (95% CI); *p*[§]
668 A>G (Q223R)					
Codominant	AA[b]	34.3 (24)	33.8 (25)	0.43	1.0
	AG	54.3 (38)	47.3 (35)		1.1 (0.54-2.33); 0.73
	GG	11.4 (8)	18.9 (14)		0.6 (0.21-1.67); 0.32
Dominant	AA[b]	34.3 (24)	33.8 (25)	0.94	1.0
	AG+GG	65.7 (46)	66.2 (49)		0.9 (0.49-1.94); 0.94
Recessive	AA+AG[b]	88.6 (62)	81.1(60)	0.21	1.0
	GG	11.4 (8)	18.9 (14)		0.5 (0.21-1.41); 0.21
Allele	A[b]	61.4 (86)	57.4 (85)	0.49	1
	G	38.6 (54)	42.6 (63)		0.8 (0.52-1.35); 0.49

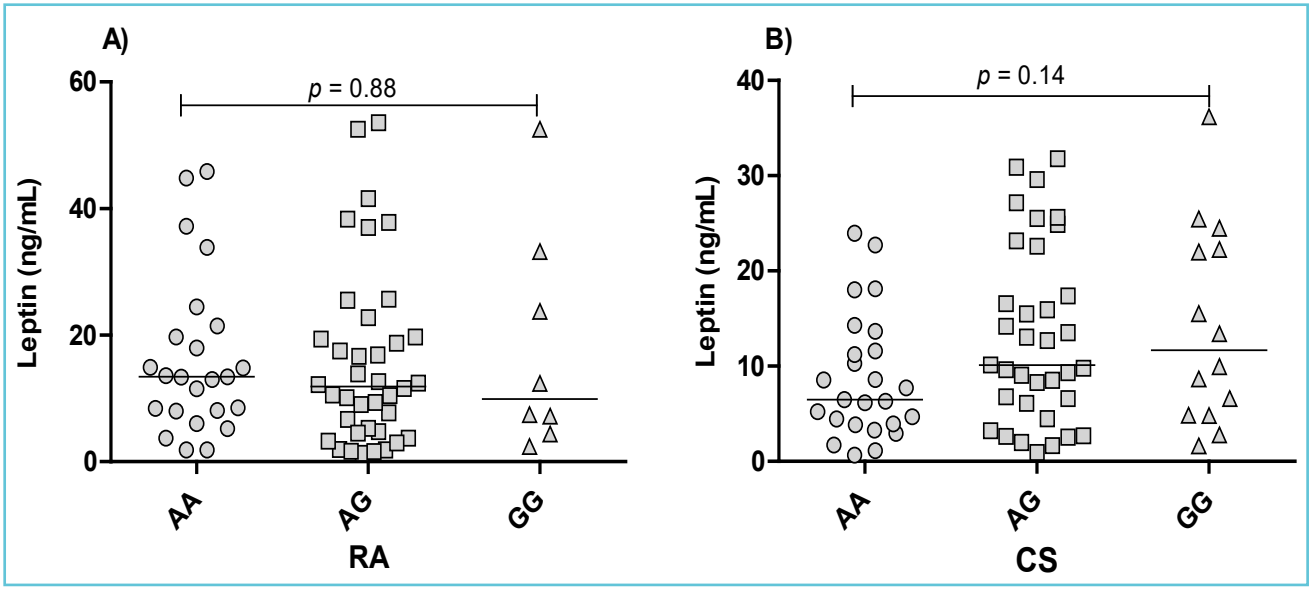
Percentages were obtained by count direct.
RA: rheumatoid arthritis; CS: control subject; OR: odds ratio; 95% CI, 95% confidence interval;
[a]p value was calculated by χ^2 test; [b] Reference category; [§]p value was calculated by logistic regression.

Figure 1 Serum leptin levels in RA patients and CS



Statistical analysis was performed using Mann–Whitney U test.

Figure 2 Comparison of circulating serum leptin levels according to *LEPR* Q223R genotype in RA patients (A) and CS (B)



Statistical analysis was performed using Mann–Whitney U test.

Table 3 Correlation between serum leptin levels and clinical characteristics of AR patients

	Leptin levels	
	r	*P*
Age (years)*	0.172	0.154
Height (kg)*	0.534	<0.001
Weight (m)*	-0.236	0.050
BMI (Kg/m^2)*	0.702	<0.001
ESR (mm/h)**	0.072	0.154
CRP (mg/dL)**	0.121	0.317
RF (UI/mL) **	0.018	0.880
DAS28*	-0.135	0.266
Disease evolution (years)*	-0.018	0.881

Evaluated with Pearson or Spearman** correlation.*
RF: rheumatoid factor; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

In both groups, the mean differences were not statistically significant.

In RA patients, serum leptin levels correlated strongly with weight, height, and BMI. No correlation was found between leptin levels and RF, CRP, ESR, disease duration and DAS28 index (Table 3).

DISCUSSION

Several studies suggest the importance of the adipose tissue as a hormone producer. These hormones are called "adipokines" which play an important role in immunity and inflammation. One of these hormones is leptin and emerging evidence indicates that this hormone acts as a proinflammatory cytokine in immune responses such as systemic lupus erythematosus, multiple sclerosis, psoriasis and RA (26). Thus, the present study attempted to find an association between the Q223R leptin receptor gene polymorphism and leptin levels in patients with rheumatoid arthritis from Western Mexico. We found that RA patients had lower weight as compared to CS. These data are interesting because Chen Y et al., reported that in males with RA, the height is inversely associated with the severity of disease and disability, and this association is independent of other factors. In the case of females, it appears that there is an association of sex and a lower height with severe disease activity and disability (27). The significantly lower weight in the RA patients is also consistent with a previous report, where weight, height, and BMI loss was significant in females with RA (27, 28). However, our results should be interpreted with caution, as most RA patients were women with very short stature as compared to the CS group. Additionally, it is notable that BMI value of patients and controls are the same.

Goldring SR et al., have argued that the loss in weight and height observed in RA patients could be explained by a generalized involvement of the bone tissue, that causes osteopenia and bone erosion (29). Several studies propose that various factors such as proinflammatory cytokines, low mobility, and a poor nutrition can promote osteopenia (30-32). This may be due to increased appetite-regulating adipokines such as leptin, as reported in certain inflammatory conditions, such as obesity or in this case in RA.

We found significantly elevated serum leptin levels in patients with RA as compared to the control subjects. These results are consistent with previous reports (4-11). Although, the values that we report in this study are higher than the healthy subjects, they are considered low as compared to other populations (16, 33), this observation may be due to differences in age, sex and ethnical background (2, 3, 33, 34). Similarly to patients with obesity and insulin resistance, the observed increase in leptin levels may be explained in part by macrophage infiltration into the adipose tissue (35-37) and not due to presence of the studied polymorphism. Further investigations are needed.

The frequencies of the allele G of the polymorphism Q223R *LEPR* gene reported in our study (0.42) are similar to those reported for Caucasians (0.41 to 0.58), and lower than that in Asians (0.81) (38). Additionally, it needs to be noted that there is a marked difference in the frequency of this allele within the Mexican population ranging between 0.33-0.39 and 0.49 for Central Mexico (39,40) and Western Mexico (25), respectively, probably explained by the increased inter-racial mixing within the population (38, 41).

In the present study, we did not find an association between LEPR Q223R polymorphisms and serum leptin levels. These results are similar to those reported by others, where no significant differences were found in leptin levels

with genotypes in healthy subjects and subjects with metabolic abnormalities (20, 21, 33). Even *in vitro* experiments have shown that the presence of this polymorphism does not affect the signaling pathway of its receptor (42).

In both groups, serum leptin levels had a strong correlation with BMI and weight, and are consistent with previous reports, which reported positive correlation between leptin levels and body composition variables in CS (43-45) and RA patients (46-48). On the other hand, in RA patients, no correlation was found between leptin levels and ESR, RF, CRP, disease duration and disease activity (DAS28). Some studies (10, 49-52) support our findings, while others have reported positive correlation between serum leptin with inflammatory markers (5-7, 9). This discrepancy could be explained by the differences in sample size, study design, and ethnic background.

CONCLUSION

Our findings suggest that in patients with RA, there is no association between the *LEPR* Q223R polymorphism and RA, despite high serum leptin levels. The elevated leptin levels may explain the low weight observed in RA patients as compared to the control subjects. Furthermore, in our study the serum leptin levels did not correlate with the studied inflammatory markers, and disease severity and duration.

REFERENCES

1. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol. 2007; 7:429-42.

2. Nakanishi T, Li R, Liu Z, Yi M, Nakagawa Y, Ohzeki T. Sexual dimorphism in relationship of serum leptin and relative weight for the standard in normal-weight, but not in overweight, children as well as adolescents. Eur J Clin Nutr 2001; 55:989-93.

3. Martin LJ, Mahaney MC, Almasy L, MacCluer JW, Blangero J, Jaquish CE, Comuzzie AG. Leptin's sexual dimorphism results from genotype by sex interactions mediated by testosterone. Obes Res 2002; 10:14-21.

4. Salazar-Páramo M, González-Ortiz M, González-López L, Sánchez-Ortiz A, Valera-González IC, Martínez-Abundis E, Balcázar-Muñoz BR, García-González A, Gámez-Nava JI. Serum leptin levels in patients with rheumatoid arthritis. J Clin Rheumatol 2001; 7:57-9

5. Bokarewa M, Bokarew D, Hultgren O, Tarkowski A. Leptin consumption in the inflamed joints of patients with rheumatoid arthritis. Ann Rheum Dis 2003; 62:952-6.

6. Lee SW, Park MC, Park YB, Lee SK. Measurement of the serum leptin level could assist disease activity monitoring in rheumatoid arthritis. Rheumatol Int 2007; 27:537-40.

7. Targónska-Stepniak B, Majdan M, Dryglewska M. Leptin serum levels in rheumatoid arthritis patients: relation to disease duration and activity. Rheumatol Int 2008;28:585-91.

8. Seven A, Güzel S, Aslan M, Hamuryudan V. Serum and synovial fluid leptin levels and markers of inflammation in rheumatoid arthritis. Rheumatol Int 2009; 29:743-7.

9. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gómez-Reino JJ, Gualillo O. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis 2006; 65:1198-201.

10. Gunaydin R, Kaya T, Atay A, Olmez N, Hur A, Koseoglu M. Serum leptin levels in rheumatoid arthritis and relationship with disease activity. South Med J 2006; 99:1078-83.

11. Yoshino T, Kusunoki N, Tanaka N, Kaneko K, Kusunoki Y, Endo H, Hasunuma T, Kawai S. Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. Intern Med 2011; 50:269-75.

12. La Cava A, Matarese G. The weight of leptin in immunity. Nat Rev Immunol 2004; 4:371-9.

13. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372:425-32.

14. Zabeau L, Lavens D, Peelman F, Eyckerman S, Vandekerckhove J, Tavernier J. The ins and outs of leptin receptor activation. FEBS Lett 2003; 546:45-50.

15. Peelman F, Van Beneden K, Zabeau L, Iserentant H, Ulrichts P, Defeau D, Verhee A, Catteeuw D, Elewaut D, Tavernier J. Mapping of the leptin binding sites and design of a leptin antagonist. J Biol Chem 2004; 279:41038-46.

16. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334:292-5.

17. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000; 404:661-71.

18. Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gómez-Reino JJ, Gualillo O. Leptin, from fat to inflammation: old questions and new insights. FEBS Lett 2005; 579:295-301.

19. Ge H, Huang L, Pourbahrami T, Li C. Generation of Soluble Leptin Receptor by Ectodomain Shedding of Membrane-spanning Receptors in Vitro and in Vivo. J Biol Chem 2002; 277:45898-903.

20. Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI. A singlenucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmeno-pausal Caucasian women. Hum Genet 2001; 108:233-6.

21. Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. J Clin Endocrinol Metab 2001; 86:4434-9.

22. Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougnères P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998; 392:398-401.

23. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, Lank E, Bottomley B, Lopez-Fernandez J, Ferraz-Amaro I, Dattani MT, Ercan O, Myhre AG, Retterstol L, Stanhope R, Edge JA, McKenzie S, Lessan N, Ghodsi M, De Rosa V, Perna F, Fontana S, Barroso I, Undlien DE, O'Rahilly S. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med 2007; 356:237-47.

24. Kimber W, Peelman F, Prieur X, Wangensteen T, O'Rahilly S, Tavernier J, Farooqi IS. Functional characterization of naturally occurring pathogenic mutations in the human leptin receptor. Endocrinology 2008; 149:6043-52.

25. Angel-Chávez LI, Tene-Pérez CE, Castro E. Leptin receptor gene K656N polymorphism is associated with low body fat levels and elevated high-density cholesterol levels in Mexican children and adolescents. Endocr Res 2012; 37:124-34.

26. Tian G, Liang JN, Wang ZY et al. Zhou D. Emerging role of leptin in rheumatoid arthritis. Clin Exp Immunol 2014; 177:557-70.

27. Chen Y, Yu Z, Packham JC, Mattey DL. Influence of adult height on rheumatoid arthritis:association with disease activity, impairment of joint function and overall disability. PLoS One 2013; 8:e64862.

28. Munro R, Capell H. Prevalence of low body mass in rheumatoid arthritis: association with the acute phase response. Ann Rheum Dis 1997; 56:326-9.

29. Goldring SR, Gravallese EM. Pathogenesis of bone erosions in rheumatoid arthritis. Curr Opin Rheumatol 2000; 12:195-9.

30. Ostrov BE. Nutrition and pediatric rheumatic diseases. Hypothesis: cytokines modulate nutritional abnormalities in rheumatic diseases. J Rheumatol 1992; 19:49–53.

31. Bacon MC, White PH, Raiten DJ et al. Nutritional status and growth in juvenile rheumatoid arthritis. Semin Arthritis Rheum 1990; 20:97–106.

32. Polito C, Strano CG, Rea L et al. Reduced bone mineral content and normal serum osteocalcin in non-steroid-treated patients with juvenile rheumatoid arthritis. Ann Rheum Dis 1995; 54:193–6.

33. Ragin CC, Dallal C, Okobia M, Modugno F, Chen J, Garte S, Taioli E. Leptin levels and leptin receptor polymorphism frequency in healthy populations. Infect Agent Cancer 2009; 4:1-6.

34. Schoof E, Stuppy A, Harig F, Carbon R, Horbach T, Stöhr W, Rascher W, Dötsch J. Comparison of leptin gene expression in different adipose tissues in children and adults. Eur J Endocrinol 2004; 150:579-84.

35. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-808.

36. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003; 112:1821-30.

37. Wellen KE, Hotamisligil GS.Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 2003; 112:1785-8.

38. Paracchini V, Pedotti P, Taioli E. Genetics of leptin and obesity: a HuGE review. Am J Epidemiol 2005; 162:101-14.

39. Guízar-Mendoza JM, Amador-Licona N, Flores-Martínez SE, López-Cardona MG, Ahuatzin-Trémary R, Sánchez-Corona J. Association analysis of the Gln223Arg polymorphism in the human leptin receptor gene, and traits related to obesity in Mexican adolescents. J Hum Hypertens 2005; 19:341-6.

40. Méndez-Sánchez N, Bermejo-Martínez L, Chávez-Tapia NC, Zamora-Valdés D, Sánchez-Lara K, Uribe-Ramos MH, Ponciano-Rodríguez G, Baptista-González HA, Ramos MH, Uribe M.Obesity-related leptin receptor polymorphisms and gallstones disease. Ann Hepatol 2006; 5:97-102.

41. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. Nat Genet 2004; 36:1312-8.

42. Stratigopoulos G, LeDuc CA, Matsuoka N, Gutman R, Rausch R, Robertson SA, Myers MG Jr, Chung WK, Chua SC Jr, Leibel RL. Functional consequences of the human leptin receptor (LEPR) Q223R transversion. Obesity (Silver Spring) 2009; 17:126-35.

43. Yadav A, Jyoti P, Jain SK, Bhattacharjee J. Correlation of adiponectin and leptin with insulin resistance: a pilot study in healthy north Indian population. Indian J Clin Biochem 2011; 26:193-6.

44. Luke AH, Rotimi CN, Cooper RS, Long AE, Forrester TE, Wilks R, Bennett FI, Ogunbiyi O, Compton JA, Bowsher RR. Leptin and body composition of Nigerians, Jamaicans, and US blacks. Am J Clin Nutr 1998; 67:391-6.

45. Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI. Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. J Clin Endocrinol Metab 1997; 82:2148-52.

46. Nishiya K, Nishiyama M, Chang A, Shinto A, Hashimoto K. Serum leptin levels in patients with rheumatoid arthritis are correlated with body mass index. Rinsho Byori 2002; 50:524-7.

47. Anders HJ, Rihl M, Heufelder A, Loch O, Schattenkirchner M. Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. Metabolism 1999; 48:745-8.

48. Gómez-Bañuelos E, Navarro-Hernández RE, Corona-Meraz F, Madrigal-Ruíz PM, Martín-Marquez BT, Pizano-Martinez OE, Aguilar-Arreola J, Perez-Cruz PJ, Macias-Reyes H, Gonzalez-Lopez L, Gamez-Nava JI, Salazar-Páramo M, Vazquez-del Mercado M. Serum leptin and serum leptin/serum leptin receptor ratio imbalance in obese rheumatoid arthritis patients positive for anticyclic citrullinated peptide antibodies. Arthritis Res Ther 2015; 17:335.

49. Anders HJ, Rihl M, Heufelder A, Loch O, Schattenkirchner M. Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. Metabolism 1999; 48:745-8.

50. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis 2005; 64:1195-8.

51. Hizmetli S, Kisa M, Gokalp N, Bakici MZ. Are plasma and synovial fluid leptin levels correlated with disease activity in rheumatoid arthritis? Rheumatol Int 2007; 27:335-8

52. Wislowska M, Rok M, Jaszczyk B, Stepień K, Cicha M. Serum leptin in rheumatoid arthritis. Rheumatol Int 2007; 27:947-54.